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Leucoagaricus lahorensis, a new species of *L.* sect. *Rubrotincti*

T. Qasim*, T. Amir, R. Nawaz, A.R. Niazi, & A.N. Khalid

Department of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore, 54590, Pakistan

*Correspondence to: *tayyaba.qasim@yahoo.com*

Abstract—*Leucoagaricus lahorensis* sp. nov. is described and illustrated based on morpho-anatomical and molecular (ITS) characteristics. The new species, which is placed in *L.* sect. *Rubrotincti*, is characterized by an umbonate to plane pileus with central obtuse orange red disc, a white to cream stipe, ascending annulus, broadly ellipsoid basidiospores, narrowly clavate to subcylindrical cheilocystidia without crystals at their apex, and straight cylindrical pileal elements.

Key words—*Agaricaceae*, macrofungi, phylogenetic analysis, Punjab

Introduction

Leucoagaricus Singer (*Agaricaceae*, *Agaricales*) is represented by more than 100 species worldwide (Kirk et al. 2008, Kumar & Manimohan 2009, Liang et al. 2010, Vellinga et al. 2010, Muñoz et al. 2012 & 2013, Malysheva et al. 2013). The genus is characterized by non-striate pileus margins, free lamellae, spores that are metachromatic in cresyl blue, and the absence of clamp connections and pseudoparaphyses (Singer 1986, Vellinga 2001). *Leucoagaricus* has been shown to be polyphyletic (Vellinga 2004).

Most *Leucoagaricus* species have been described from temperate regions in North America and Europe, and little is known about the genus from tropical areas (Liang et al. 2010). Representatives of *L.* sec. *Rubrotincti* form moderately fleshy basidiomata with a context that does not change color when damaged and usually a red brown, pinkish ochre, olive, or orange pileus with radially arranged hyphae (Singer 1986). Basidiospores are characterized by a smooth surface and incomplete or absent germ pores.

Lepiotaceous fungi occur widely in Punjab at lower elevations. Previously only two *Leucoagaricus* species, *L. leucothites* (Vittad.) Wasser and *L. serenus*

(Fr.) Bon & Boiffard, have been reported from Pakistan (Ahmad et al. 1997). During macrofungal surveys conducted in the monsoon seasons of 2011–13, a species was encountered that differs macro- and microscopically from other species of *Leucoagaricus*. A detailed description and illustrations of this novel species are presented.

Materials & methods

Morpho-anatomical analysis

Basidiomata of *Leucoagaricus lahorensis* were carefully collected, photographed, and vouchered. The dried specimens were characterized morphologically using terminology of Vellinga & Noordeloos (2001). Colours were designated according to Munsell (1975). Basidiocarps were sectioned and placed in 5% KOH and stained with phloxine for microscopic observation. Colour reactions were also noted in Melzer's reagent. At least eighty basidiospores from two collections and twenty each of basidia, cheilocystidia, and pileal and stipe elements were measured using an ocular micrometer. The following abbreviations are used: avl = average length, avw = average width, Q = length/width of basidiospores, avQ = the mean Q of all basidiospores. Drawings of these microscopic features were made using a camera lucida attached to a compound microscope. The holotype and paratype specimens have been deposited in the Herbarium, Department of Botany, University of the Punjab, Lahore, Pakistan (LAH).

DNA extraction, PCR amplification, & DNA sequencing

DNA was extracted by the CTAB method (Bruns 1995). The internal transcribed spacer region (ITS) was amplified using universal primers ITS4 and ITS1F (Gardes & Bruns 1993). PCR conditions were as described in Gardes & Bruns (1993). The amplified products were directly sequenced using the same pair of universal primers (Macrogen, Korea). The nucleotide sequence comparisons were performed through Basic Local Alignment Search Tool (BLAST) network services using National Centre for Biotechnology Information (NCBI), USA database. For phylogenetic analysis and sequence alignment, sequences of closely related species were retrieved from GenBank. Sequence alignment was done in Muscle Multiple sequence alignment and phylogenetic analysis was performed using Molecular Evolutionary Genetic Analysis (MEGA 5) software. Phylogenetic analyses were made by Maximum Likelihood method using Jukes Cantor Model (Tamura et al. 2011). The nucleotide sequences were submitted to GenBank.

Results

Leucoagaricus lahorensis Qasim, Amir & Nawaz, **sp. nov.** Figs 1, 2

MycoBank MB808587

Differs from *Leucoagaricus rubrotinctus* by its thin-walled larger broadly ellipsoid to amygdaloid spores and narrowly subclavate to sub-cylindrical cheilocystidia.

Type: Pakistan, Punjab, Lahore, grounds of University of the Punjab, 217 m a.s.l., gregarious on soil, 3 September 2012, A.N. Khalid T25 (Holotype, LAH 10042012; GenBank KJ701794).

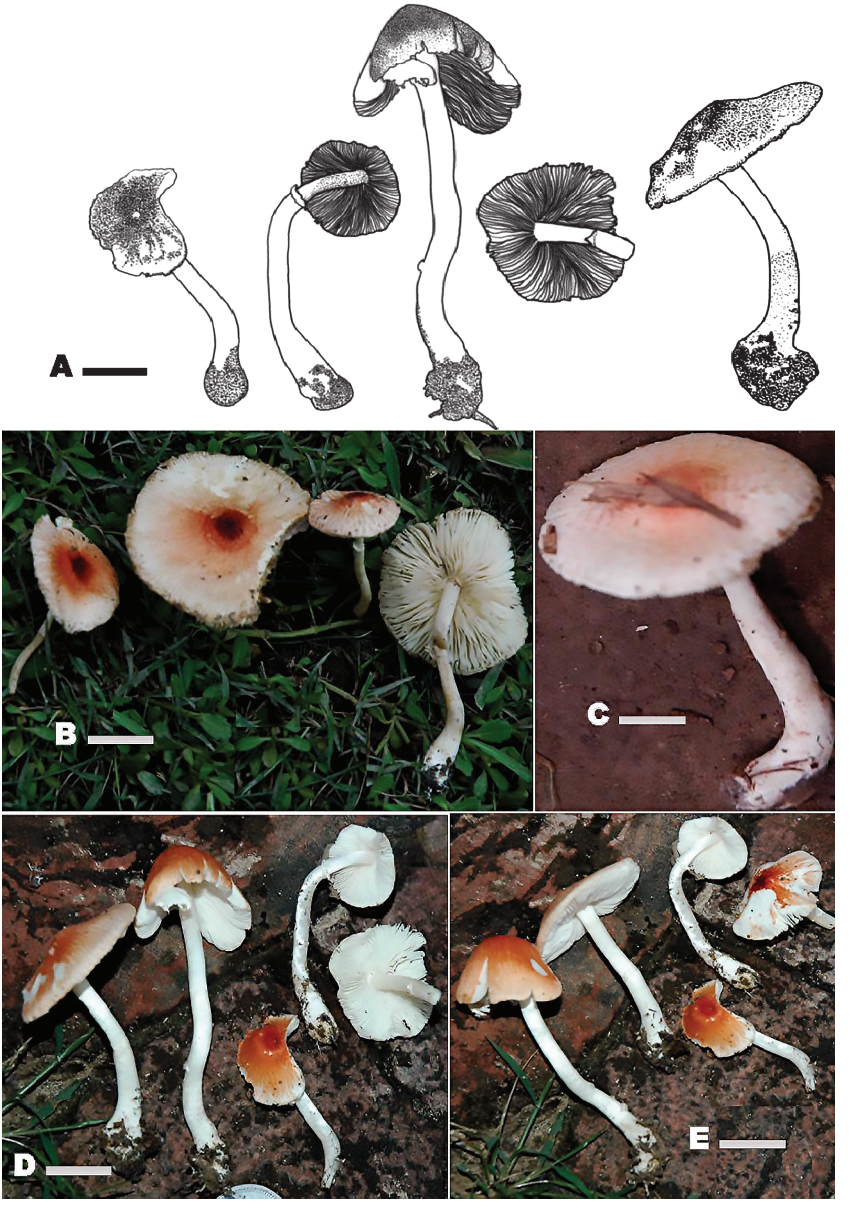

Fig. 1: *Leucoagaricus lahorensis:* basidiomata A–E (A & D–E, Holotype, B, T38; C, CM36) in their natural habitat. Scale bar: A = 13 mm, B & C = 18 mm, D–E = 20 mm.

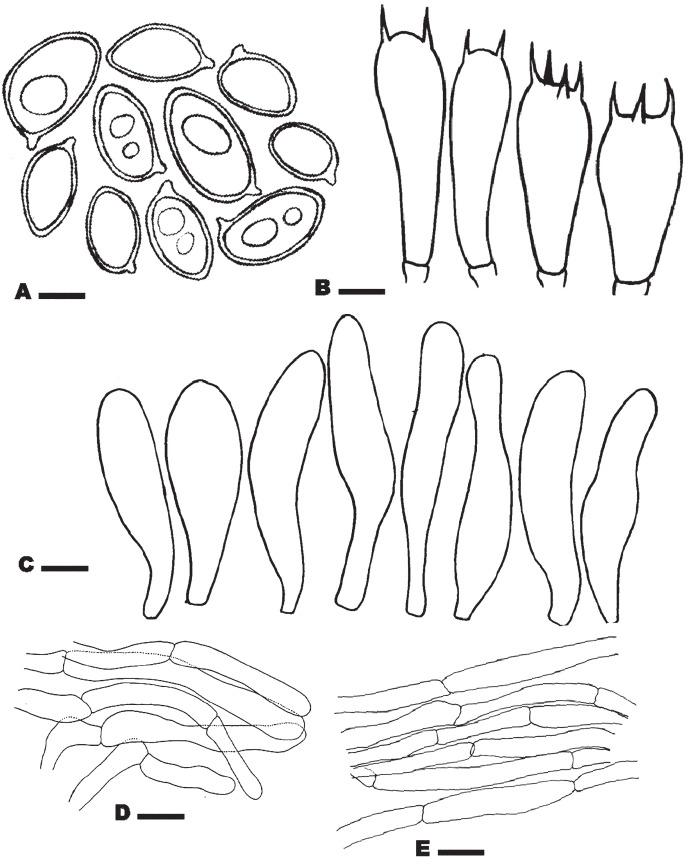

Fig. 2: *Leucoagaricus lahorensis* (Holotype):
A, basidiospores; B, basidia; C, cheilocystidia; D, stipe covering; E, pileus covering.
Scale bar: A–B = 6 µm, C = 9 µm, D = 47 µm, E = 30 µm.

Etymology: from the type locality Lahore, provincial capital of Punjab, Pakistan.

Basidiomata 3.8–7.3 cm tall. Pileus 2.5–5.5 cm in diameter, at first umbonate, then becoming plane with deflexed margins at maturity; central disc obtuse, dark reddish brown (10 R 3/10); surface squamulose, with radially arranged reddish brown fibrils, becoming sparse and light brown (7.5R5/16) towards margins over a white to cream background; context white, fleshy; margins entire, with cracks developing at maturity. Lamellae free, close, thin, membranous, white to cream (5YR9/2), edges entire; lamellulae in 3–4 tiers. Stipe 2.9–5.6 × 0.3–0.7

cm, central, equal to sub-equal, cylindrical, slightly attenuating towards pileus, with shiny white surface (5YR9/2), hollow, smooth; base slightly bulbous. Annulus present, membranous, white to cream, ascending, superior. Volva absent. Taste and odour not recorded.

Basidiospores [80/4/4], (7.3)8–10.6(10.9) × (6.1)6.4–7.6(7.8) µm, avl × avw = 8.7 × 6.7 µm, Q = 1.2–1.4, avQ = 1.3, broadly ellipsoid to ellipsoid, occasionally amygdaloid, hyaline with greenish tinge in KOH, thin-walled, smooth, apiculate, guttulate, dextrinoid in Melzer's reagent; germ pore absent. Basidia (17.2)17.5–18.9(19.1) × (10.1)10.6–11.6(11.8) µm, avl × avw = 17.8 × 11.2 µm, thin-walled, hyaline in KOH, 2–4-spored, broadly clavate to vesicular, smooth. Cheilocystidia (33.7)34.1–38.7(40) × (8)9.1–10.5(11.3) µm, avl × avw = 24.2 × 10.7 µm, hyaline in KOH, thin-walled, narrowly clavate to sub-cylindrical, smooth. Pleurocystidia not present. Stipitipellis made of cells 11.8 – 23.6 µm diam., avw = 17.6 µm, thin-walled, hyaline in KOH, parallel in arrangement, septate, unbranched; clamp connections absent. Pileipellis hyphae 5.7–11.6 µm (av = 7.5 µm) diam, light brown in colour. Clamp connections absent.

Additional material examined: **PAKISTAN, Punjab**, Lahore, University of the Punjab, 217 m a.s.l., gregarious on rich loamy soil, 18 August 2011, A.N. Khalid MCR84 (LAH 8482011; GenBank KJ701797); on grounds of botanical garden, 21 September 2012, A.N. Khalid & Tayyaba Qasim T38 (LAH 10052012; GenBank KJ701794); Changa Manga Forest, on rich soil, 28 July 2013, A.R. Niazi & Tooba Amir CM36 (LAH 2481336; GenBank KJ701795).

Phylogenetic analysis

ITS-nrDNA amplification produced fragments of 700 -742 bp. The initial BLAST analysis revealed the maximum similarity of *L. lahorensis* with *L. rubrotinctus*. The top 100 sequences in BLAST analysis that represent *Leucoagaricus* and other related taxa were retrieved for phylogenetic analysis. From the final data set comprising 48 sequences, 47 represented the ingroup, while *Cystolepiota seminuda* (AY176350) was selected to root the tree (Fig. 3).

The final alignment comprised 763 nucleotides, of which 316 characters were conserved and 324 were parsimony informative sites. Gaps were treated as missing data. Tree topologies remained the same in all three phylogenetic analyses: Maximum Likelihood (ML), Maximum Parsimony, and Nearest Neighbour Joining).

In the ML phylogenetic tree two different *Leucoagaricus* sections were obtained: *L.* sect. *Rubrotincti* and *L.* sect. *Piloselli* (Fig. 3). The taxa that clustered together in different sections also have morphological similarities. All *L. lahorensis* sequences fall in *L.* sect. *Rubrotincti,* along with the sequences of *L. littoralis*, *L. rubrotinctus, L. sublittoralis* sensu Reid, *L. vassiljevae*, *L. wichanskyi*, and other *Leucoagaricus* species of Vellinga (2001, 2004).

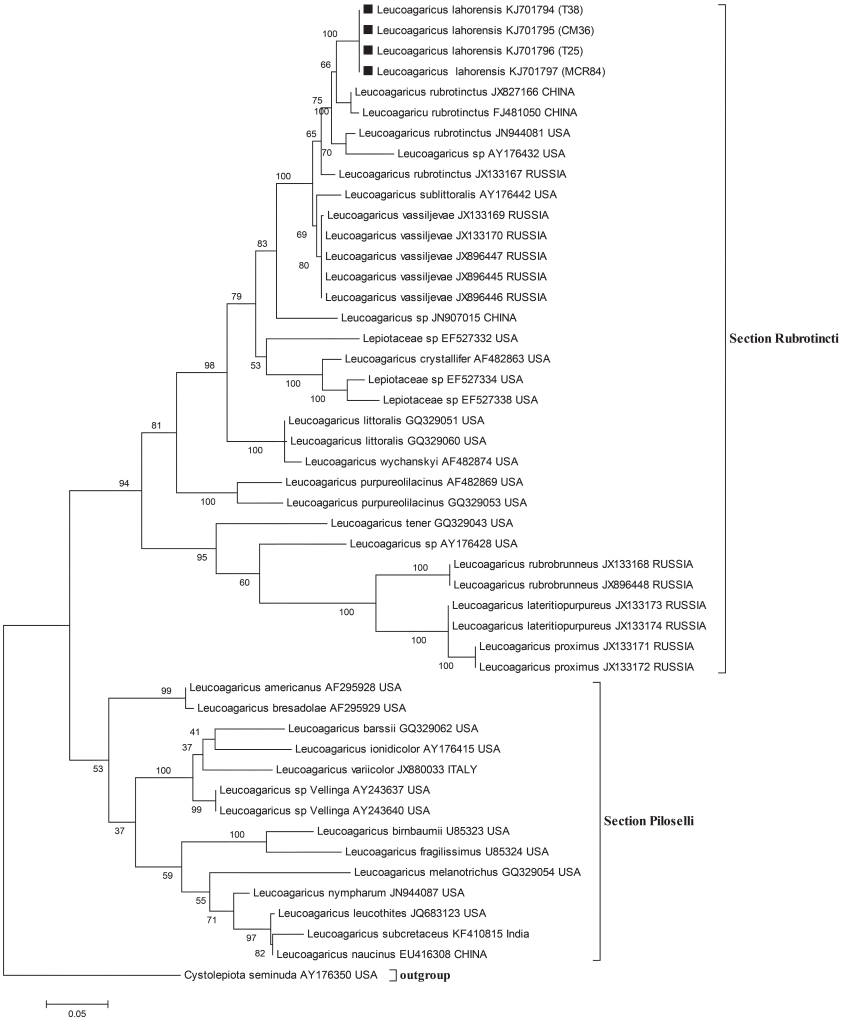

Fig. 3: Phylogram obtained from ITS region of nrDNA. The phylogenetic relationship of *Leucoagaricus lahorensis* was inferred from nrITS sequences using Maximum Likelihood method. Bootstrap values more than 50% are present above branches. *Leucoagaricus lahorensis* sequences are indicated by ■.

Discussion

Basidiomata representing *Leucoagaricus lahorensis* were collected frequently from Lahore during the August and September rainy seasons of 2011–13. This study is based on four collections representing the new taxon: the holotype T25 and MCR84, T38, and CM36. Preliminary morphological analysis suggested that all four collections represented the same species, which our molecular analyses confirmed. The ITS-nrDNA based phylogenetic tree also supports *L. lahorensis* as separate from all other *Leucoagaricus* spp. for which ITS data are available in Genbank.

Leucoagaricus lahorensis is characterized by a reddish brown pileus covering with white background, radially arranged fibrils becoming sparse towards margins, close lamellae with entire margins, a white annulus, broadly ellipsoid basidiospores (occasionally with an amygdaloid apex), narrowly clavate to sub-cylindrical cheilocystidia without apical crystals, and cylindrical pileal elements with clavate to narrowly clavate terminal ends.

Our ML phylogenetic tree revealed two *Leucoagaricus* sections that agree with those recognized by Singer (1975). All species in *L.* sect. *Rubrotincti* are characterized by a reddish brown pileus covering, ellipsoid spores, tetrasporic basidia, no colour change upon bruising, and a negative ammonia reaction (Singer 1948, 1986; Bon 1993; Vellinga 2001).

All *L. lahorensis* sequences clustered together in *L.* sect. *Rubrotincti* with 72% bootstrap support. *Leucoagaricus lahorensis* forms a sister clade with Chinese collections tentatively called *L. rubrotinctus* (66% bootstrap support). The morphology of these Chinese collections is not known; *L. rubrotinctus* (Peck) Singer is an eastern North American species with an orange-red radially fibrillose pileus (Vellinga 2001). *Leucoagaricus lahorensis* is distinguished by its white annulus, broadly ellipsoid spores, and subclavate to cylindrical cheilocystidia.

Another closely related species is *L. vassiljevae* E.F. Malysheva et al. Both species share the approximately same size of pileus (4.5 cm diam for *L. vassiljevae*), red brown radially arranged fibrils on a white background, and broadly ellipsoid dextrinoid basidiospores. However, the large spores (8–13 × 5–6 μm), clavate cheilocystidia, and longer stipe (5–13 cm) of *L. vassiljevae* distinguish it from *L. lahorensis*.

Leucoagaricus sublittoralis (Kühner ex Hora) Singer resembles *L. lahorensis* in its pinkish brown pileus with white background, 4-spored basidia, and dextrinoid spores, but its broadly ellipsoid spores and cheilocystidia without crystals render it a different taxon. Furthermore, *L. sublittoralis* is found in clay, loamy soils (Vellinga 2001), while *L. lahorensis* is found in loamy soils.

Leucoagaricus rufosquamulosus T.K.A. Kumar & Manim. also shows similarities with *L. lahorensis* based on brownish red pileus covering, but the spores with germ pores set it apart from *L. lahorensis*. *Leucoagaricus glabridiscus* (Sundb.) Wuilb. has similarly long spores (10.3 µm) (Vellinga 2001) but is distinguished from *L. lahorensis* by its 1–2 tiers of lamellulae.

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